Physiologically-Based Pharmacokinetic/Pharmacodynamic Modeling:

Preliminary Evaluation and Case Study for the N-Methyl Carbamate Pesticides

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TABLE OF CONTENTS

EXECUTIVE SUMMARY

The Food Quality Protection Act of 1996 requires EPA to consider potential human health risks from all pathways of dietary and non-dietary exposures to more than one pesticide acting through a common mechanism of toxicity. In 2001, EPA established the N-methyl carbamate pesticides as a common mechanism group based on their structural characteristics and also similarity and shared ability to inhibit acetylcholinesterase (AChE) by carbamylation of the serine hydroxyl group located in the active site of the enzyme. EPA has not determined what method or methods will be used to estimate cumulative risk for this common mechanism group.

EPA is in the early stages of developing a strategy for incorporating physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) models into its cumulative risk assessments. PBPK/PD models are very powerful tools that can account for anatomic structure and physiological and biochemical processes. They can be used to estimate internal exposure dose or concentrations of parent compounds and/or active metabolites at the target site(s) and also toxicologically relevant effects. Typically, inhibition of AChE is fairly rapid (within hours) for members of the N-methyl carbamate common mechanism group. The time dependant relationship between exposure and effect for this group make the N-methyl carbamates a good case study to aid the Agency in developing its strategy for using PBPK/PD models in cumulative risk assessments.

The following document provides the preliminary model structure for two separate PBPK/PD models being developed in addition to the biological basis for their structure. These models are being developed in separate programming languages (ACSL and MCSim). Six simulations using are provided that show types of relevant output that can be generated by PBPK/PD models. Theses simulations include results for *in silico* experiments for a single chemical at starting values; a single chemical with adjustments to the AChE regeneration rate and gastrointestinal parameters; two exposures to a single chemical separated by either 1 hour or 4 hours; and one exposure each to two different chemicals 4 hours apart. Development of PBPK/PD models are resource and data intensive. This document details types of *in vivo* and *in vitro* data that are desirable for development and evaluation of PBPK/PD models for individual N-methyl carbamates and also the cumulative assessment group as a whole. The critical steps (defining the model purpose; biological characterization; mathematical description, computer implementation, and parameter analysis and quality of model fit) in evaluating the quality of PBPK/PD models are also discussed.

The current document is considered a research effort that is a work-in-progress; model development is still on-going. The level of refinement afforded during the model development and evaluation phases will be directly related to the amount of relevant and appropriate pharmacokinetic and pharmacodynamic data available. EPA expects further scientific review in the future as the case study develops further and as the strategy for incorporating PBPK/PD models in cumulative risk assessments matures.

LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
AUC	Area under the curve
CAG	Cumulative assessment group
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FQPA	Food Quality Protection Act
GI	Gastro-intestinal
LOAEL	Lowest-Observed-Adverse-Effect Level
NHEERL	National Health and Environmental Effects Laboratory
NERL	National Exposure Research Lab
NOAEL	No-Observed-Adverse-Effect Level
OP	Organophosphate pesticide
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
PBPK	Physiologically-based pharmacokinetic (typically refers to models)
PBPK/PD	Physiologically-based pharmacokinetic/pharmacodynamic (typically refers to models)
PD	Pharmacodynamic
PK	Pharmacokinetic
RBC	Red blood cells
SAP	Scientific Advisory Panel

I.   BACKGROUND AND SCOPE

In 1996, passage of the Food Quality Protection Act (FQPA) imposed upon the Office of Pesticide Programs (OPP) the requirement to consider potential human health risks from all pathways of dietary and non-dietary exposures to more than one pesticide acting through a common mechanism of toxicity. At each step in the development of its cumulative risk assessment guidance and methodology, OPP has solicited scientific peer review. Specifically, the FIFRA Scientific Advisory Panel (SAP) has reviewed OPP's Cumulative Guidance (FIFRA SAP 1999, 2000) and the many aspects of the cumulative risk assessment for the organophosphates pesticides (OPs; see USEPA, 2002a). The Cumulative Guidance (USEPA, 2002a) describes several methods which could be used for performing cumulative hazard assessment. Some of these include use of effect levels from toxicology studies [e.g., no-observed-adverse-effect (NOAELs) and/or lowest-observed-adverse-effect levels (LOAELs)]; benchmark dose modeling (USEPA, 2000b); and also physiologically-based pharmacokinetic/pharmacodynamic models (PBPK/PD). Each of these methods are considered reasonable approaches to doing cumulative hazard assessment. As discussed in the Cumulative Guidance (USEPA, 2002a), the level of refinement for each cumulative risk assessment will be depend on several factors, included among these is the availability of adequate and appropriate data for the particular common mechanism group of interest. The FIFRA SAP has previously encouraged OPP to consider using PBPK models (FIFRA SAP 2001, 2002) in developing cumulative risk assessments. EPA is currently developing a draft strategy for utilizing pharmacokinetic data in cumulative risk assessments. As part of this draft strategy, a collaborative research effort is underway at EPA's National Health and Environmental Effects Laboratory (NHEERL) and National Exposure Research Lab (NERL) along with Rory Conolly of the CIIT Centers for Health Research to develop a case study using PBPK modeling for multiple pesticides with a common mechanism of action. This case study is being developing with the N-methyl carbamate pesticides.

In 2001, EPA established the N-methyl carbamate pesticides as a common mechanism group based on their structural characteristics and also similarity and shared ability to inhibit acetylcholinesterase (AChE) by carbamylation of the serine hydroxyl group located in the active site of the enzyme (USEPA, 2001b). The N-methyl carbamate pesticides are, therefore, subject to cumulative risk estimation under the FQPA (1996). OPP is in the early stages of developing its cumulative risk assessment of this common mechanism group and expects to have a preliminary cumulative risk assessment for the relevant AChE-inhibiting members of this class to be available to the public by spring of 2005. The AChE inhibitory effects of N-methyl carbamates is reversible generally within hours--although the time to recovery is chemical-dependent. The time course of effects can be very complicated for multi chemical risk assessments when the time to effect and/or the time to recovery varies substantially among chemicals. OPP has not yet determined the method or methods it will use to estimate the cumulative risk to this common mechanism group. Consistent with past practice for single chemical assessments and the principles outlined in the Cumulative Guidance, OPP may first perform a screening level assessment to evaluate those pesticides and exposure scenarios which may or may not be likely to contribute to the cumulative risk

prior to development of a more refined assessment for the contributing pesticides and/or exposure scenarios. OPP acknowledges that consideration of the pharmacokinetics and pharmacodynamics of AChE inhibition at the target site(s) and time to recovery are important factors in developing the preliminary cumulative risk assessment for the N-methyl carbamate pesticides.

As discussed below, PBPK and physiological based pharmacodynamic (PBPD) models offer great advantages in risk assessment, such as the ability to incorporate pharmacokinetic and mechanistic information, to consider the assumptions of dose-additivity, and to evaluate intra- and inter-species extrapolation. There are, however, practical implications and considerations in a regulatory setting such as the availability of appropriate data for developing and evaluating the model and also quality assurance/quality control. The Agency is currently drafting a strategy for utilizing pharmacokinetic data and PBPK/PD models in cumulative risk assessments. The purpose of the current SAP review is to consider the on-going case study for N-methyl carbamate pesticides to aid the Agency in the development of this draft strategy.

The current evaluation considers both conceptual and technical aspects of performing cumulative risk assessment using a PBPK/PD model. The ability to directly consider mechanistic information, such as time to recovery data, is highlighted. The case study includes model simulations for two theoretical chemicals with toxicological and physical-chemical properties consistent with those for N-methyl carbamate pesticides. The current document does not consider actual exposure scenarios or estimate cumulative risk. The PBPK/PD models described in this document are still under development. Parameter estimation and sensitivity analysis are not discussed here. This document also does not consider the application of uncertainty factors or the application of the FQPA 10X Factor for infants and children. This document does, however, consider possible ways of incorporating exposure information and considers types of relevant information that could be output from a PBPK/PD model.

II.   INTRODUCTION

Pharmacokinetic models range from simple empirically based models that describe observed data to more complex PBPK models that can be used to predict outcomes and extrapolate from one set of exposure conditions to another based upon an understanding of the underlying biology. A PBPK model is a quantitative description (typically with differential equations) of the biological structures and processes that control pharmacokinetic (PK) behavior in an organism (i.e, the effect of the body on the absorption, distribution, metabolism, and excretion of a chemical). PBPK modeling differs from classical compartmental PK modeling in this focus on the biological determinants of PK behavior. PBPK models simulate the events between the external dose and the internal exposure of the chemical to a target site. PBPD models address the events from the internal dose at the target site to the response observed (i.e., the effects of the chemical on the body), e.g., inhibition of AChE. PBPK/PD models are used to establish a linkage between PK behavior and the toxicological or biological effect of a chemical on the body, such as inhibition of AChE. Thus, while classical empirical modeling is useful for interpolation between data points, *a well developed PBPK/PD model can be used to simulate toxicological outcomes for a variety of different exposure conditions* (e.g., different test species, exposure routes, chemical concentrations, or metabolizing capacity).

PBPK/PD models have the potential to consider internal exposure concentrations at the site(s) of action for a single chemical and its toxicologically active metabolite(s) and/or multiple chemicals and their respective metabolites. Dose additivity is EPA's default assumption when evaluating the joint risk of chemicals that are toxicologically similar and act at the same target site (USEPA, 2001a). In cases where multiple chemical species are considered in the PBPK/PD modeling, the impact of possible additive or non-additive interactions between the different chemical species can be described. For example, sites of biotransformation and/or binding to enzymes can be described. Specifically, the PBPK/PD models can provide time course quantitative outputs of concentration, amount, or changes in endogenous enzymes and thus the models can track the PK behavior and pharmacodynamic (PD) outcome of mixtures.

Consideration of how the biology described in the model changes with age, sex, species and/or other factors can guide development of these models. Development and use of these models requires knowledge of organism specific and chemical specific biologic processes. An understanding of the parameters that govern the pharmacokinetics is also necessary. Proper development and use of these models often requires examination of existing data, model formulation and testing leading to more specific data requirements, which in turn leads to model refinement. These capabilities allow PBPK/PD models to serve two somewhat different roles. First, the models can play key roles in the laboratory study of pharmacokinetics and mechanism of action. This role of PBPK/PD models is particularly powerful when model development and laboratory experiment are conducted in an iterative, mutually supportive manner. Models help identify key data which are lacking, elucidate important events in the chain leading to toxicity, and also identify and quantify the uncertainty. For example, PBPK/PD models may inform us about nonlinearities in high to low dose

extrapolation and about interspecies scaling factors that would not have been apparent without a quantitative, mechanistic perspective.

A second role of PBPK/PD models is in the development of risk assessments. PBPK/PD models developed from an adequate supporting database that have been tested and evaluated, and also demonstrate reasonable ability to predict the behavior of datasets not used during model development, can be used for partial or complete replacement of the default assumptions used in risk assessment (e.g.,inter- and intra-species extrapolation factors or route-to-route extrapolation). EPA has previously used PBPK models to estimate the toxicologically relevant dose for dichloromethane (USEPA, 1995; Anderson et al, 1987) and vinyl chloride (USEPA, 2000c; Clewell et al, 1995a, b).

The capacity of PBPK/PD models to explicitly consider mechanistic data, to estimate exposure concentrations at the site(s) of action, and to describe the pharmacokinetic behavior of mixtures motivate EPA's interest in this type of modeling and specifically in the development of a case study with the N-methyl carbamate pesticides. As stated above, the Agency is developing a draft strategy for utilizing PBPK/PD models in cumulative risk assessments. A more detailed description of this strategy will be provided at a later date following consideration of the comments from the SAP and further progress has been made on the case study.

III.   CASE STUDY: N-METHYL CARBAMATE PESTICIDES

A.   Cumulative risk assessment and PBPK/PD models

Based on the risk assessment paradigm described by the National Research Council (1983, 1994), risk is made up of exposure and hazard components. The discrepancy between actual and predicted risk is minimized to the extent that these two factors are well-characterized. This minimization serves the public health by providing the soundest possible guidance for setting exposure standards. Society as a whole is in turn well served when the stringency of exposure standards is aligned as closely as possible with the actual magnitude of the health risk. This alignment helps to ensure the efficient allocation of scarce resources. In contrast, risk assessments based largely on default assumptions, while expected to be health-protective, provide little assurance that exposure standard stringency and the actual magnitude of the health risk are well aligned with each other.

In the specific case of cumulative risk assessment for the N-methyl carbamate pesticides, AChE inhibition is considered to be the toxicologically relevant regulatory endpoint. No PBPK or PBPD models for N-methyl carbamates have been published to date (October, 2003). Such models have been described for several OPs that describe the inhibition of AChE (Gearhart et al, 1990 and 1994, Timchalk et al, 2002). The OP models describe the key anatomical, physiological, and biochemical factors that control OP pharmacokinetics and the transport of the AChE-inhibiting chemical to AChE. These models thus describe the PK mechanisms of OPs as well as the inhibition and regeneration of AChE. The existence of PBPK/PD models for OPs have facilitated the development of the preliminary model for N-methyl carbamates. For the on-going case study, eventually, PBPK/PD models will be prepared for individual N-methyl carbamate pesticides and then linked together to predict AChE inhibition following exposure to multiple N-methyl carbamate pesticides.

Both time-course and dose-response behaviors for AChE inhibition can be tracked by PBPK/PD models with arguably greater confidence than is possible with empirical models that do not incorporate the physiological and mechanistic detail that characterize PBPK/PD models or with default approaches which do not consider any chemical or exposure specific data or information. When used for risk assessment purposes, this increased confidence in model-generated predictions compared to empirically-based and/or default-based approaches relates to a reduction in the overall uncertainty about risk estimates. These models thus serve the goal of moving towards more accurate prediction of risk without any relaxation of concern for protection of the public health.

It should be recognized that PBPK/PD modeling in support of cumulative risk assessment for N-methyl carbamates can be expected to reduce but not to eliminate uncertainty. PBPK/PD model structures and parameter values have associated degrees of uncertainty some of which cannot be readily eliminated. A

key consideration in the overall evaluation of this exercise will thus be the degree to which PBPK/PD modeling increases confidence in the final assessment relative to the confidence that would be obtained with a less sophisticated approach.

The present document considers preliminary work on the methods for estimating exposure at the site(s) of action and subsequent AChE inhibition for the N-methyl carbamate pesticides . This document does not consider relevant environmental exposure scenarios from food, water, and/or residential and non-occupational settings. It should, however, be noted that a well-developed PBPK/PD model is sufficiently flexible to consider various types and combinations of exposure and co-exposure scenarios appropriately separated in time. These models should also be sufficiently flexible to consider discreet exposure scenarios for a single person or distributions of exposures for many people. OPP is still actively considering which method or methods are most appropriate for use in estimating the cumulative risk for these pesticides. The degree to which OPP considers results from the PBPK/PD modeling effort will depend, in part, on the availability of appropriate PK and PD data but also on the resources required to perform computer simulations for specific exposure scenarios.

B.   Preliminary pharmacodynamic description of acetylcholinesterase inhibition by N-methyl carbamate pesticides

EPA established the N-methyl carbamate pesticides as a common mechanism group based on their structural characteristics and also similarity and shared ability to inhibit AChE by carbamylation of the serine hydroxyl group located in the active site of the enzyme (USEPA, 2001b). This inhibition results in accumulation of acetylcholine at a nerve synapse or neuromuscular junction. This inhibition can result from interaction between the parent N-methyl carbamate pesticide or AChE-inhibiting metabolites with the enzyme, AChE. Continued accumulation of the neurotransmitter acetylcholine may result in the overstimulation of cholinergic pathways in the central and peripheral nervous systems and possibly to the expression of cholinergic signs and symptoms such as nausea, gastrointestinal distress, vomiting, tremors, paralysis and depression of respiratory function.

Generally, AChE-inhibiting chemicals compete with the acetylcholine for binding to the enzyme (AChE). As more AChE-inhibiting chemical binds with the enzyme, the acetylcholine is subject to slower or less hydrolysis and its activity is prolonged. The following outlines the basic process of AChE-inhibition for a single N-methyl carbamate pesticide.

1.   There is a certain amount of AChE in each tissue and a certain amount is synthesized to keep this level at a near physiological steady-state (K_s). This is a basic physiological process independent of any foreign chemicals entering the system.

2. A certain amount of enzyme is degraded (K_d). This also reduces the amount of free enzyme available to perform its normal physiological function. When no inhibitor is present this degradation process is balanced by the synthesis described above. However in the presence of inhibitor the formation of the complex can be thought of as another stress that reduces the amount of enzyme available for normal physiological function. This reduces the activity of the enzyme on its normal physiological substrate, acetylcholine at the neurologic site.

3. Inhibitors, such as the N-methyl carbamates, enter the system and reduce the amount of free enzyme by forming a complex with the enzyme. The enzyme that is complexed with the AChE-inhibiting chemical is no longer available to perform its normal physiological activity leading to the build up of acetylcholine. (Each N-methyl carbamate pesticide has a unique rate constant for the formation of the complex with the enzyme, K_i).

4. The enzyme-inhibitor complex in turn reacts to result in a break down of the AChE-inhibiting chemical and a return or regeneration of free enzyme. This process is also governed by a chemical specific rate constant, K_r. The period of inhibition varies for different compounds and is generally dependent upon the rate of regeneration. Because the period of inhibition is often brief (due to rapid regeneration) the whole process has been dubbed as 'reversible'.

Figure 1 summarizes this process. The "released metabolite" in Figure 1 represents the N-methyl carbamate that is broken down. Note that each carbamate has its own specific rate constants for the process. Any number of N-methyl carbamates can interact at same time or at any time with the free AChE.

Figure 1. Schematic diagram of N-methyl carbamates binding to AChE.
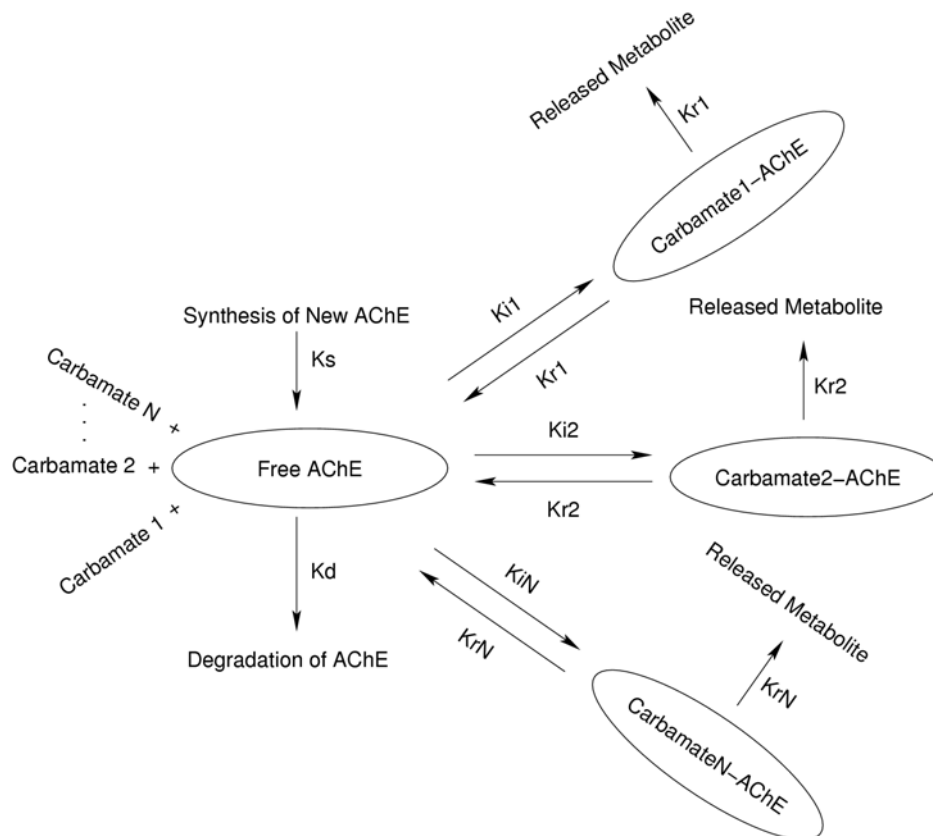
Where:
Ace$_x$ is the amount of AChE (: mol) in compartment *x*
INce$_{xj}$ is the amount (: mol) complex of AChE and inhibitor *j* in compartment *x*
K_s is zero-order rate of enzyme synthesis
K_d is the first-order rate of enzyme degradation (hr^{-1})
K_{ij} is the bimolecular rate of inhibition for *j*th inhibitor
K_{rj} is the first-order rate of regeneration for *j*th complex
Carbamate N is AChE active chemical, parent compound or metabolite
Released metabolite is a Non-AChE active metabolite

The following differential equations represent the mass balance for the Figure 1.

Equation 1
$$\frac{dAce_x}{dt} = K_s - Ace_x \times \left(K_d + \sum_j K_{Ij} \times C_{jx} \right) + \sum_j K_{rj} \times INce_j$$

Equation 2
$$\frac{dINce_{xj}}{dt} = Ace_x \times K_{Ij} \times C_{jx} - K_{rj} \times INce_j$$

Where:
Ace_x is the amount of AChE (: mol) in compartment x
$INce_{xj}$ is the amount (: mol) complex of AChE and inhibitor j in compartment x
K_s is zero-order rate of enzyme synthesis
K_d is the first-order rate of enzyme degradation (hr^{-1})
K_{Ij} is the bimolecular rate of inhibition for j^{th} inhibitor
K_{rj} is the first-order rate of regeneration for j^{th} complex
subscript x indicates tissue compartment,
subscript j indicates the identity of the inhibiting chemical

Thus, the total amount of active enzyme is equal to the amount present in the system minus the amount degraded minus the amount forming a complex with the inhibitor plus the amount regenerated after the enzyme breaks down or metabolizes the inhibitor.

Cumulative risk assessments consider risk from multiple pesticides. Therefore, the PD component of the PBPK/PD model needs to include the capacity to consider potential mixture effects. More than one compound can act in combination at any of the steps outlined above. The simplest interaction would be simply adding the inhibition caused by each compound. In such cases, depending, upon the specific rate constants, different chemical molecules would each contribute to enzyme inhibition. It might be possible however that interaction would involve competition between the various chemicals for binding with the enzyme. If data suggest that interactions between the N-methyl carbamates other than dose-additive ones are observed, these can and will be included in the modeling efforts.

C.   Preliminary pharmacokinetic description of N-methyl carbamate pesticides

As described in previous sections, PBPK models describe the disposition of the foreign chemical throughout the body and within the tissues. For purposes of this assessment the N-methyl carbamates are modeled as being predominately metabolized in the liver with secondary metabolic sites in the kidney and brain compartments.

Parameters for PBPK models include three distinct types of data: physiological, chemical-specific, and parameters for determining the stochastic behavior of model. The physiological data are independent of the chemical being modeled and refer to such things as organ volumes and blood flows. Some chemical-specific parameters are partition coefficients, metabolic rate constants, and coefficients for protein binding. Parameters for determining the stochastic behavior of model, such as inter-individual variances, are discussed below in Sections III.G and III.H.

The specific compartments considered in the modeling are selected based on information available for exposure, toxicology, and metabolic profile to a particular chemical and potential active metabolites. Distribution within, between and among organs, tissues, and fluid is modeled according to compartmental volumes, blood flow rates, and blood tissue partitioning. The body volume is determined for each animal species, based on sex and age. The compartment volumes are then calculated as a percentage of the body volume. Generally, each model has equations to explicitly describe the arterial and venous blood, the lung, the liver, and kidney. Other organs are lumped together within two compartments referred to as rapidly and slowly perfused tissues. Organs of toxicological interest such as neurologic organs are also included as explicit organs (explicit means that the organ has its own equations and is not included in one of the lumped compartments). All of the flows of the compartments (organs) must add up to 100% of the cardiac output. Table 1 provides selected organs and necessary governing parameters for the N-methyl carbamate model.

Table 1. Selected organs and parameters relevant for PBPK modeling for the N-methyl carbamate pesticides

Organs	Parameters
Arterial and Venous Blood	cardiac output, arterial blood volume, venous blood volume, binding constants; cholinesterase levels, rate constants for interaction with cholinesterase
Liver	liver volume, tissue to blood partition coefficients for all chemicals (parents and metabolites) metabolism rate constants; cholinesterase levels, rate constants for interaction with cholinesterase
Stomach and intestine	absorption parameters
Kidney	kidney volume, tissue to blood partition coefficients for all chemicals (parents and metabolites) metabolism rate constants
Brain	brain volume, tissue to blood partition coefficients for all chemicals (parents and metabolites) metabolism rate constants; cholinesterase levels, rate constants for interaction with cholinesterase
Rapidly and Slowly perfused tissues	organ volume, tissue to blood partition coefficients for all chemicals (parents and metabolites)
Lung	respiratory rate, tissue to blood partition coefficients for all chemicals (parents and metabolites) and where appropriate, blood to air partition coefficients
Appropriate tissues	Equilibrium binding constants for binding to proteins, etc.

Absorption involves entry of a drug or chemical into the body. A chemical may enter directly into the gastro-intestinal (GI) tract via gastric gavage, from ingestion of food, or from "non-dietary" ingestion. The basic GI model has a stomach and intestine that are simulated with rate and bolus ingestion into the stomach, flow from the stomach to the intestine and from the intestine to intestinal elimination. In addition there is flow from the stomach and the intestine to the liver via portal blood. There is no bile flow from the liver to the intestine and no lymph flow or pool. Systemic arterial blood and portal venous blood are input into the liver. Mathematical expressions used to describe absorption into the GI tract are presented in Equation 3.

The stomach has the jth chemical input by bolus ingestion (a plug of food or drink) and rate ingestion (food or drink input over time), with chemical output to the liver via portal blood and to the intestine. The equation for the rate of change of the jth chemical in the stomach is:

Equation 3
$$\frac{dA_{ST_j}}{dt} = \frac{dA_{BIG_j}}{dt} + \frac{dA_{RIG_j}}{dt} - K_{ABS,ST,PB_j} A_{ST_j} - K_{ST,IN_j} A_{ST_j} ,$$

Where:
A_{st} is the amount of chemical in the stomach
A_{Big} is the amount of chemical resulting from a bolus ingestion
A_{rig} is the amount of chemical resulting from a "rate" ingestion (gradual over time rather than a bolus)
$K_{abs, St,PB}$ is the rate constant for absorption from the stomach into the portal blood
$K_{st, in}$ is the rate constant for transfer from stomach to intestine

The rate of change of the jth chemical in the intestine is given by the rate of input from the stomach to the intestine and the rate of output to the liver via portal blood, and to the feces. The equation is:

Equation 4
$$\frac{dA_{IN_j}}{dt} = K_{ST,IN_j} A_{ST_j} - K_{ABS,IN,PB_j} A_{IN_j} - K_{IN,FEC_j} A_{IN_j}$$

Where:
A_{st}, $K_{st, in}$ are as previously defined
A_{in} is the amount of chemical in the intestine
$K_{abs,in pb}$ is the rate constant for absorption from the intestine into the portal blood
$K_{in, FEC}$ is the rate constant for fecal elimination

The most commonly used PBPK models assume each organ to be homogenous and thus the fluid contained in the vascular, interstitial or extracellular, and the intracellular regions are all combined into one. This implies that the transfer of a chemical across the membrane, i.e., capillary wall and cell membrane, is very rapid compared to the tissue perfusion rate. Under this condition, the permeability across the membrane is assumed to be very large. Therefore, the slowest or rate-limiting step in the process of drug distribution must be its delivery by the circulatory flow. The typical mass balance differential equation describing this is:

Equation 5

$$dA_{j,i}/dt = V_i\, dC_{j,i}/dt = (Q_i(C_{j,a} - C_{j,vi}) - dM_{j,i}/dt)$$

where:
$A_{j,i}$ is the amount of the jth chemical in the ith organ
$C_{j,i}$ is the concentration of jth chemical in the ith organ
t is time
Q_i is the arterial blood flow to the ith organ
$C_{j,a}$ is the concentration of jth chemical in the arterial blood
$C_{j,vi}$ is the concentration of the jth chemical in the venous blood leaving the ith organ
$dM_{j,i}/dt$ is the rate of metabolism of the jth chemical in the ith organ
V_i is the volume of the ith organ

Essentially, this equation describes the transport and metabolic transformation of the chemical into and within the tissue. Under the assumptions of a well-stirred compartment, the instantaneous concentration of a substance in a tissue or organ is the difference between the concentration entering the organ and that leaving the organ adjusted by any metabolic processes also eliminating the chemical.

Under the assumed conditions that the organ is homogenous or well-stirred the venous blood leaving the organ is in equilibrium with the organ as described by:

Equation 6

$$C_{j,i}/C_{j,vi} = R_{j,i}$$

$R_{j,i}$ is referred to as the tissue to blood partition coefficient. This value, determined from a variety of computational and laboratory methods, is governed by a number of thermodynamic properties of the chemical and the tissue of the organ. In the simplest cases it represents a ratio of solubilities of the chemical in the tissue to blood. The above equation then is transformed to:

Equation 7
$$dA_{j,i}/dt = V_i\, dC_{j,i}/dt = (Q_i(C_{j,a} - C_{j,i}/R_{j,i}) - dM_{j,i}/dt)$$

The integral of the above equation results in the concentration at time, t.

The expression for metabolism can take any number of forms including Michaelis-Menten, first order, or second order. For the case of the N-methyl carbamates Michaelis-Menten and first order kinetics are employed for the various metabolic processes. Equations such as the ones above are also written and employed for each of the metabolites of the jth chemical. Further, in the appropriate tissues, the equation also include the metabolic terms to account for the transformation of the chemical caused by the cholinesterases (this has been described in previous sections).

The liver is described with similar equations with terms that account for absorption from the stomach and intestine as described previously. Other organs such as the skin and lung also have input terms as appropriate.

For purposes of the N-methyl carbamate pesticides, the model must represent multiple chemicals in some combination and even with simultaneous exposure. The power of PBPK/PD models is in the ability to use it to represent the biologic and physical process that go on within the body. Because the model mathematically describes the physical, chemical, and physiological processes it can be configured to account for the affect of multiple chemical exposure.

D.   Computer implementation

The general modeling strategy described above will be implemented in two separate modeling efforts, implemented in different languages. This activity provides a quality control check on the modeling software and the coding of the model, in that outputs of the two models given the same input should be similar. Divergence of the two model outputs would indicate improper coding in at least one of the models. Since two languages are being used that differ in syntax and how the model code is structured, it is unlikely that a coding error would be made similar enough in both programs that it would go undetected (i.e., both program outputs would be the same). Moreover, the capabilities of the two modeling languages differ, and there are features unique to each program that add to the overall ability to develop and test the model.

The descriptions of the two models follows:

1. Model 1: Use of Exposure Related Dose Estimating Model (ERDEM)

EPA's NERL has developed the Exposure Related Dose Estimating Model (ERDEM) as a platform for the application of PBPK and PBPK/PD models. The heart of ERDEM (USEPA, 2002c) is a PBPK model that simulates the absorption, distribution, metabolism, and elimination of chemicals in mammalian systems.

Simulated chemicals are introduced into the physiological system by any of several routes including injection, ingestion, inhalation, and/or dermal absorption. The ERDEM system is contains a large set of potential compartments and processes, with over 30 physiological compartments such as arterial and venous blood, brain, skin (surface and dermis), fat, intestine, kidney, liver, rapidly and slowly perfused tissues, lung, stomach, and intestine. Any given model is derived by selecting those compartments and processes that are most applicable to the kinetics of the chemical(s) and endpoint of interest. Figure 2 is a diagrammatic depiction of the pharmacokinetic model that was developed for the N-methyl carbamates.

ERDEM is programmed in the Advanced Continuous Simulation Language (ACSL). Model specific parameter values are entered into ERDEM based upon the physiological, biological, and biochemical modeling data specific to the chemical and/or scenario of interest (USEPA, 2002c). Any PBPK model, including ERDEM, is made up of a series of the differential equations which describe the rates of inflow, distribution, metabolism, or outflow of a chemical and various metabolites in each separate biological compartment. For the application of cholinesterase inhibiting compounds such as the N-methylcarbamates, ERDEM has been expanded to include a PD component. This PD component is designed to describe the effect of these compounds on the cholinesterase enzymes as described in the previous section.

ERDEM consists of the following: An ACSL-based model engine and a Power Builder Front End.   Both of these components will be made available to the public as executables from EPA's Office of Research and Development (ORD)-NERL. However at present time the front end has not been updated to include simulations for AChE-inhibiting chemicals. An executable ACSL command file that includes the AChE inhibition component can be provided to interested individuals or groups by EPA's ORD-NERL. The user is advised to run the model using ACSL command files rather than a front end. No special software is required by the user. An ACSL software license is only needed to recompile the code and cannot be provided by EPA. However ERDEM should require no additional recompilation of code to run the model as described in the document.

Inputs

Bolus Dose Ingestions

Rate Ingestions

Intraperitoneal Injection

Intramuscular injection

Skin Surface Water

Bolus Dose Injections

Infusions

$K_{ST,IN}$

$K_{IN,FEC}$

ST   Stomach

IN   Intestine

Intestinal Elimination

0.00

SP   Spleen 0.00

$K_{ST,PB}$ $K_{IN,PB}$

Spleen Metabolites

QB_{sp}

Portal Blood

LV   Liver

Liver Metabolites

QB_{LV}

CR Carcass

Carcass Metabolites

QB_{CR}

KD   Kidney

Kidney Metabolites

QB_{KD}

Kidney Elimination

FT   Fat

Fat Metabolites

QB_{FT}

SL Slowly Perfused

Slowly Perfused Tissue Metabolites

QB_{SL}

RP Rapidly Perfused

Rapidly Perfused Tissue Metabolites

QB_{RP}

DR Derma

QB_{DR}

BR   Brain

Brain Metabolites

QB_{BR}

VB   Venous

Open Chamber Inhalation

Open Chamber Exhalation

QA

PU   Static Lung

CC   Closed Chamber Inhalation

QB

Lung Metabolites

AB   Arterial

There are N chemicals modeled. Liver, Kidney, Fat, Carcass, Brain, Slowly Perfused, Rapidly Perfused Tissue and Spleen. The Static Lung, and Lung Tissue are modeled with binding, elimination, and metabolism.

There are up to K metabolites of each of the N chemicals. Each metabolite is one of the N chemicals. There is binding in the Arterial Blood and Venous Blood.
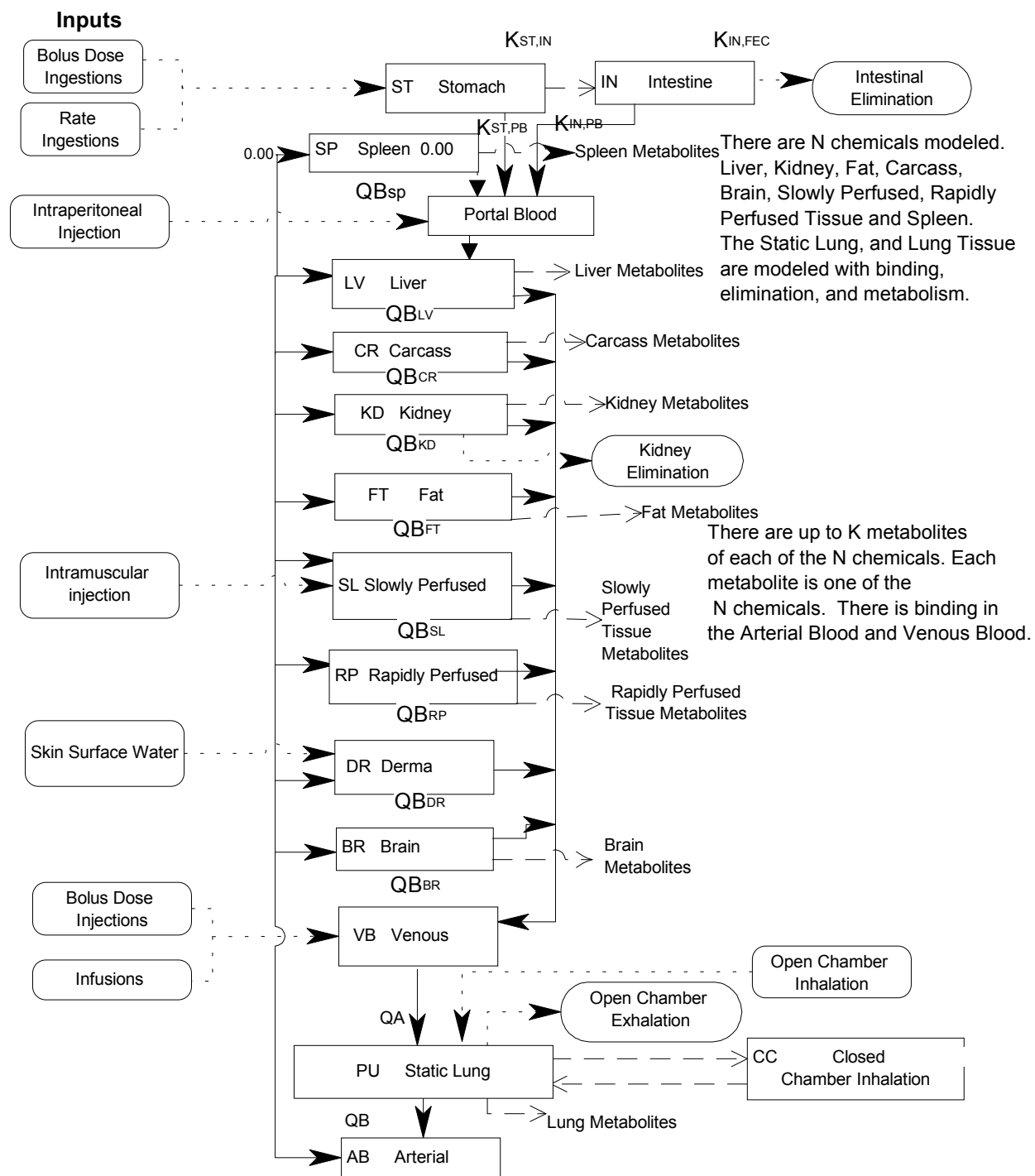
Figure 2.   Schematic diagram of PBPK model for *N* chemicals in ERDEM.

2. Model 2: PBPK development using MCSim Language

A second model is being developed in the MCSim language. Figure 3 shows a schematic of part of that model, for a single N-methyl carbamate that has two active metabolites. MCSim is an open-source statistical modeling package initially developed by Frederick Bois for the application of modern Monte Carlo statistical methods in complex nonlinear models. Since MCSim includes a sublanguage for describing dynamic models in terms of their component differential equations and typical time-varying inputs, it has been particularly valuable in the application of Markov chain Monte Carlo (MCMC) methods to estimating Bayesian posterior distributions for parameters of PBPK/PD models.

Dynamic models in MCSim are written in an algebraic language. Model specification includes predefining all the parameters for the model, declaring all the variables whose dynamics are governed by differential equations, declaring all the variables whose values need to be output, specifying input variables whose values will be determined by special functions that provide for periodic or episodic inputs, as well as the differential equations for the model. This model specification file is translated by the MCSim software into the C programming language, and then compiled and linked to libraries that provide routines for integrating the differential equation system, carrying out the required Monte Carlo simulations (USEPA, 1996 and 1997), and doing the input and output functions. The resulting executable file is then run with specially formatted input files that can change parameter values and specify the nature of the desired simulation, whether it is a numerical integration of the differential equation system, a Monte Carlo simulation of parameter variability or uncertainty, or a MCMC estimate of Bayesian posterior distributions for model parameters.

MCSim models are portable at several levels. At the lowest level, since MCSim itself is open-source, unlike ACSL, and since open source c-language compilers are available for almost all computing platforms (e.g., UNIX, Microsoft Windows, and Apple OS-X), models can be distributed as model source, and recompiled and run with little additional cost on the part of reviewers. Compiled models are also executable files, and can be run without any additional software (though the executables are specific to particular operating systems and computing hardware). Thus, the compiled models can be distributed and their behavior evaluated without the installation of additional software.

At the present time, the MCSim is still under development. As the case study for the N-methyl carbamates is developed further, the computer code will be provided to the public at a later date.

Figure 3. Schematic diagram of PBPK model for a single N-methyl carbamate with two active metabolites in MCSim. (Red coded compartments contain terms for AChE inhibition.)

E.   Types of output from by PBPK/PD models

As described above PBPK/PD models are very powerful tools that can help account for anatomic structure and physiological and biochemical processes. They can be used to evaluate the disposition of the chemicals and their metabolite in the body and any relevant PD outcome(s). The types and formats of the output from PBPK/PD models can vary and should be related and defined by the purpose of the model. Below provides a list of possible output that may be relevant to examining the N-methyl carbamate pesticides. Each of the different examples provide information about single or multiple pesticide exposure and relate to examining PD issues of AChE inhibition or the estimated exposure dose or concentration at the target site(s). This list is not meant to be exhaustive but rather provide examples of possible outputs.

1.   Area under the curve (AUC) for AChE inhibition which equals or exceeds a particular level. Following exposure to one or more N-methyl carbamates, the AUC can be calculated for AChE inhibition that exceeds a pre-determined level, 10% AChE for example.

2.   Duration for AChE inhibition which equals or exceeds a particular level. Following exposure to one or more N-methyl carbamates, the duration for AChE inhibition that exceeds a pre-determined level, 10% AChE for example, can be estimated.

3.   AUC for AChE inhibition over a pre-determined duration of time, such as 1 hour, 4 hours, or 24 hours.

4.   Peak level of AChE inhibition, particularly in red blood cell (RBC) or brain.

5.   AUC for concentration(s) of active AChE-inhibiting chemicals can be calculated.

6.   Peak concentration(s) of active AChE-inhibiting chemicals can be estimated in the target tissue(s).

7.   Time to ½ peak concentration(s) of active AChE-inhibiting chemicals can be estimated in the target tissue(s).

Some of these example outputs are shown in the simulations below.

### F.	Illustrative simulations and example output

Previous sections of this document have provided the biological basis and general structure of the PBPK models under development for the N-methyl carbamate pesticides. The following discussion describes simulations under six different example conditions which illustrate the PBPK/PD modeling approach and its usefulness. The first three simulations consider exposure to a single chemical at a 'starting' set of parameters followed by changes in AChE regeneration rate and GI absorption. Simulations 4 and 5 consider the impact of time to recovery and time of exposure on AChE for two exposures to a single chemical. Simulation 6 considers single exposure to two different chemicals. All the simulations presented here were performed using ERDEM as previously described. The two chemicals described below have toxicological and physical-chemical properties consistent with one or more N-methyl carbamate pesticides. However, Chemical-1 and Chemical-2 do not represent actual pesticide chemicals and are used here only for illustrative purposes. Similarly the exposures simulated below do not represent actual or real exposure levels. The exposure amounts were selected arbitrarily for purposes of illustration only.

#### 1.	Simulation 1: Single oral gavage exposure to Chemical-1 at starting parameter values

Results from Simulation 1 are shown in Table 2 and Figures 4 and 5. In this simulation, Chemical-1 was administered *in silico* at 10 mg/kg via gavage to male rats.

Table 2. Example outputs for Chemical-1 in the brain and venous blood following oral 10 mg/kg exposure by gavage*

Example Outputs	Brain	Venous Blood
Peak Inhibition (%)	36.02	32.58
AUC at 1 Hr/24 Hr (mg-Hr/L)	19.1/34.8	2.1/3.5
Duration of Inhibition above 10% (Hours)	5.25	4.85
Peak Concentration (mg/L)	34.05	6.39
Time to ½ of Peak Concentration (Hours)	0.3	0.15

*The original values are k_r=0.6, Stomach to Portal Blood Rate=13.65, Stomach to Intestine Rate=2.18, and Intestine to Portal Blood Rate=0.044; Male Sprague-Dawley rats, mean body weight 284g.

Figure 4. Comparison of simulation results for brain AChE inhibition in Chemical-1 using starting parameter values with actual experimental data for a N-methyl carbamate pesticide.
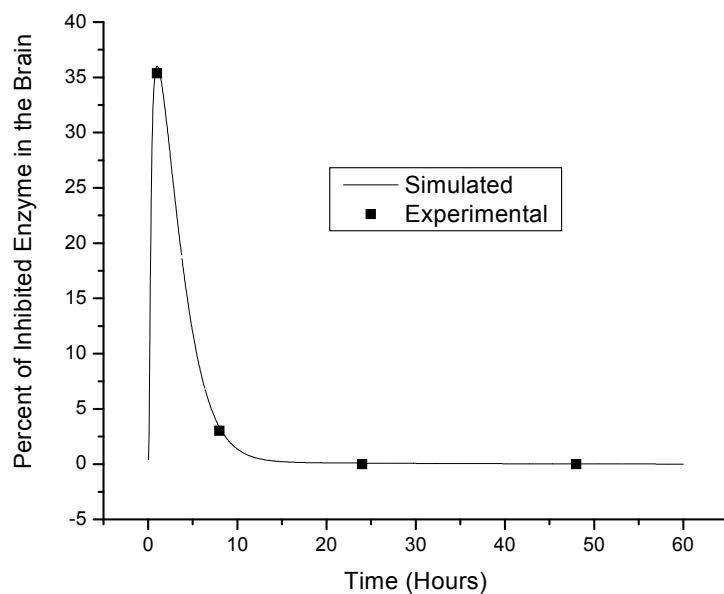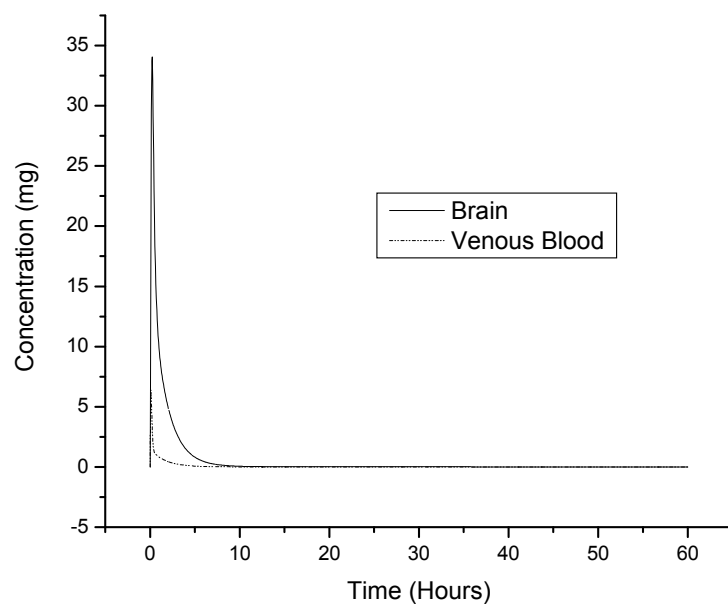
Figure 5. Comparison of simulation output for exposure concentrations of the Chemical-1 with starting parameter values in the venous blood and brain.

2. Simulation 2: Single oral gavage exposure to Chemical-1 reduction in AChE regeneration rate

The impact of reducing enzyme inhibition regeneration rate from 0.6 /hr to 0.3 /hr was tested *in silico* while maintaining the same GI absorption conditions (Table 3 and Figure 6). Compared to Table 2, the results of Simulation 2 show that reducing the regeneration resulted in an increase in peak inhibition and increase in duration of AChE inhibition over 10%. It should be noted, that peak exposure concentrations in brain and venous blood remained unchanged. Relative to the starting values, peak inhibition, although greater, is reached less rapidly and the decline in inhibition is evidently more protracted.

Table 3. Example outputs for Chemical-1 in the brain and venous blood following oral 10mg/kg exposure by gavage with lower AChE regeneration rate*

Example Outputs	Brain	Venous Blood
Peak Inhibition (%)	43.43	39.27
AUC at 1 Hr/24 Hr (mg-Hr/L)	19.1/34.8	2.1/3.5
Duration of Inhibition above 10% (Hr)	8.65	8.15
Peak Concentration (mg/L)	34.05	6.39
Time to ½ of Peak Concentration (Hr)	0.3	0.15
GI Absorption, Dose = 2.837	Amount (mg)	Time to 99.9% (Hr)
Total from Stomach to Portal Blood	2.45	0.45
Total from Intestine to Portal Blood	0.36**	> 60.0

*The regeneration rate is k_r=0.3. The original GI values are Stomach to Portal Blood Rate=13.65, Stomach to Intestine Rate=2.18, and Intestine to Portal Blood Rate=0.044; Male Sprague-Dawley rats, mean body weight 284 g.
**0.03 mg remained unabsorbed in the intestine after 60 hours.

Figure 6. Comparison of simulation results for brain AChE inhibition in Chemical-1 using a lower AChE regeneration rate with actual experimental data.
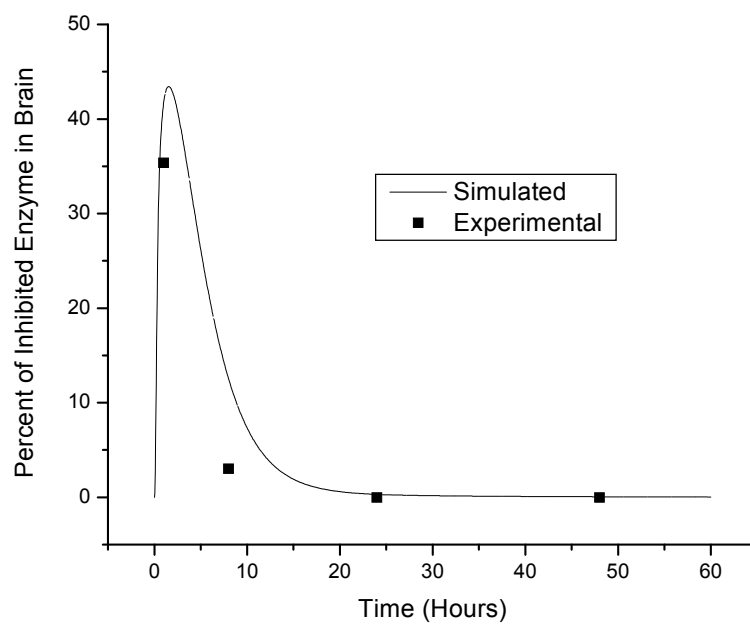
3. Simulation 3: Single oral gavage exposure to Chemical-1 reduction in GI absorption parameters

In Simulation 3, changes were made to the previously used GI absorption rates (Table 4 and Figure 7). These modifications included decreases to the stomach to portal blood rate from 13.65 to 4.55 and the stomach to intestine rate from 2.18 to 0.218, and an increase in the intestine to portal blood rate from 0.044 to 0.10. Realistically, these values for these parameters values will be dependent on the vehicle used to deliver the gavage dose, the physicochemical properties of the chemical of interest and dietary conditions during dosing.

Under these modified absorption conditions, peak inhibition in brain (30.72%) and venous blood (27.39%) was reduced when compared with prior absorption and AChE regeneration rate conditions (Table 2) and under modified AChE regeneration rate conditions (Table 3).
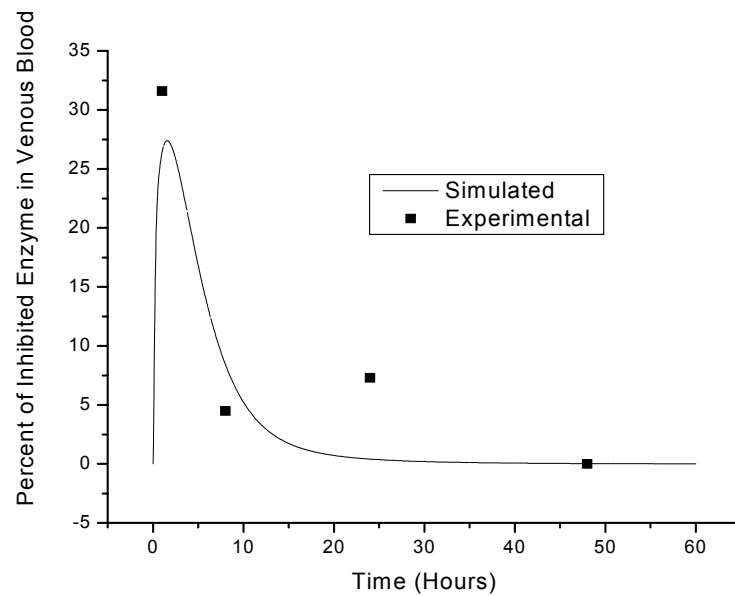
Table 4: Example outputs for Chemical-1 in the brain and venous blood following oral 10mg/kg exposure by gavage reduced GI values and modified regeneration rate.*

Example Outputs	Brain	Venous Blood
Peak Inhibition (%)	30.72	27.39
AUC at 1 Hr/24 Hr (mg-Hr/L)	11.50/23.1	1.28/2.32
Duration of Inhibition above 10% (Hr)	7.65	7.05
Peak Concentration (mg/L)	18.7	2.9
Time to ½ of Peak Concentration (Hr)	0.45	0.2
GI Absorption Dose=2.837 mg	Amount (mg)	Time to 99.9% (Hr)
Total from Stomach to Portal Blood	1.92	0.7
Total from Intestine to Portal Blood	0.92**	46

*The regeneration rate is k_r=0.3. The modified GI values are Stomach to Portal Blood Rate=4.55, Stomach to Intestine Rate=0.218, and Intestine to Portal Blood Rate=0.1; Male Sprague-Dawley rats, mean body weight 284 g.
**There was 0.023 mg still unabsorbed in the intestine after 60 hours.

Figure 7. Comparison of simulation results for blood AChE inhibition in Chemical-1 using a lower AChE regeneration rate and reduced GI parameter values with actual experimental data.

4. Simulation 4 : Two oral gavage exposures to Chemical-1 administered 1 hour apart

Real world exposure conditions are very complicated–from different chemicals, routes, and media. PBPK/PD models as described here can simulate complicated exposure profiles. For Simulation 4, two 10 mg/kg oral gavage exposures were administered *in silico* one hour apart (Table 5; first value in each case is the value for only one exposure with second value being the value resulting from two exposures):

Table 5. Example outputs for Chemical-1 in the brain and venous blood following oral (gavage) 10 mg/kg exposures at zero hour and one hour

Example Outputs	Brain	Venous Blood
	Single/Dual-1hr	Single/Dual-1hr
Peak Inhibition (%)	36.02/56.26	32.58/52.74
AUC at 1 Hr/24 Hr (mg-Hr/L) (top/bottom)	19.1/34.8 19.1/77.1	2.1/3.5 2.1/7.8
Duration of Inhibition above 10% (Hours)	5.25/7.4	4.85/7.05
Peak Concentration (mg/L)	34.05 2nd Peak 44.44	6.39 2nd Peak 7.44
Time to ½ of Peak Concentration (Hours)	0.3 2nd Peak 0.5	0.15 2nd Peak 0.2

The results in Table 5 show that when the second exposure is at one hour after the first one, the peak inhibition is increased by a little over 50%, The AUC at 24 hr is more than doubled, the duration of the inhibition is above 10% for about 40% longer and the peak concentration is increased by 20-25 % with the second exposure.

5. **Simulation 5 : Two oral gavage exposures to Chemical-1 administered 4 hours apart**

Table 6 shows the modeled results if the dual exposures to Chemical-1 are simulated to be within four hours of one another. For this case the peak percent inhibition and concentrations are lower because (and more similar to those resulting from just one exposure) the chemical has been mostly cleared by the time the second exposure occurs. The duration of inhibition above 10% is increased by about two hours and the AUC at 24 hour is still close to the higher value recorded for the one hour difference in exposures.

Table 6. Example outputs for Chemical-1 in the brain and venous blood following oral (gavage) 10 mg/kg exposures at zero hour and four hours

Example Outputs	Brain	Venous Blood
	Single/Dual-4hr	Single/Dual-4hr
Peak Inhibition (%)	36.02/43.13	32.58/39.87
AUC at 1 Hr/24 Hr (mg-Hr/L) (top/bottom)	19.1/34.8 19.1/70.6	2.1/3.5 2.1/7.1
Duration of Inhibition above 10% (Hr)	5.25/9.55	4.85/9.15
Peak Concentration (mg/L)	34.05 2nd Peak 35.8	6.39 2nd Peak 6.58
Time to ½ of Peak Concentration (Hr)	0.3 2nd Peak 0.35	0.15 2nd Peak 0.15

Figure 8. Simulation results for brain AChE inhibition in Chemical-1 where *in silico* exposures occurred at hour 0 and 4. (Points represent actual experiment data from a N-methyl carbamate exposed only at hour 0).
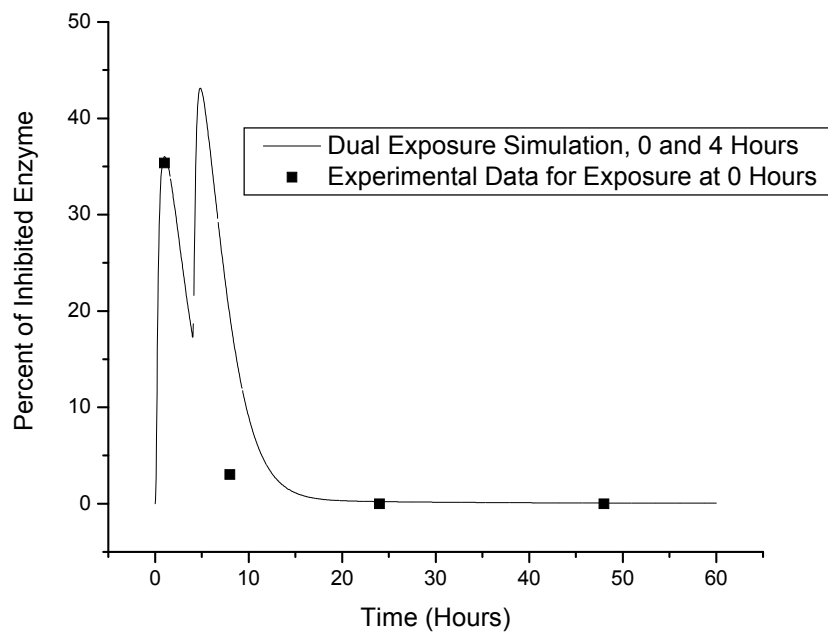
6. Simulation 6 : One oral gavage exposures to Chemical-1 and one oral gavage exposure to Chemical-2 4 hours apart

Simulation 6 illustrates the use of the model to examine the impact of exposure to two chemicals. Here the exposure to two different N-methyl carbamates was simulated within four hours of each other. The second chemical was set to have slower elimination in the liver by metabolic clearance. The maximum rate for this reaction was set to be 1/10th of the rate for the first chemical. Table 7 shows a summary of the results:

Table 7. Example outputs for Chemical-1 and Chemical-2, exposed at time zero, and a similar chemical, 1/10th of the Vmax, exposed at four hours, in the brain and venous blood

Example Outputs	Brain	Venous Blood
Peak Inhibition (%)	36.02 2nd Peak 54.79	32.58 2nd Peak 50.80
AUC at 1 Hr/24 Hr (mg-Hr/L),Chem 1 AUC at 5 Hr/28 Hr (mg-Hr/L),Chem 2	19.1/34.8 27.78/223.5	2.1/3.5 3.21/22.50
Duration of Inhibition above 10% (Hr)	25.6	24.75
Peak Concentration (mg/L)	34.05 2nd Chem Peak 40.2	6.39 2nd Chem Peak 7.07
Time to ½ of Peak Concentration (Hr)	0.3 2nd Chem 1.25	0.15 2nd Chem 0.2

As shown in Table 7, the peak inhibition increases by 50% following exposure to Chemical-2. The duration increases by a factor of almost three compared to a single chemical exposure (Table 2). The AUC 24 hours after the exposure increased by more than a factor of 6 from Chemical 1 to Chemical 2. Thus the critical factors with the chemical with the lower Vmax is the longer duration of inhibition above 10% and the significant increase in the AUC 24 hours after the exposure. Figure 9 shows the results on inhibition in the brain.

Figure 9. Simulation results for brain AChE inhibition where *in silico* exposure to Chemical-1 occurred at hour 0 and *in silico* exposure to Chemical-2 occurred at hour 4. (Points represent actual experiment data from a N-methyl carbamate exposed only at hour 0).
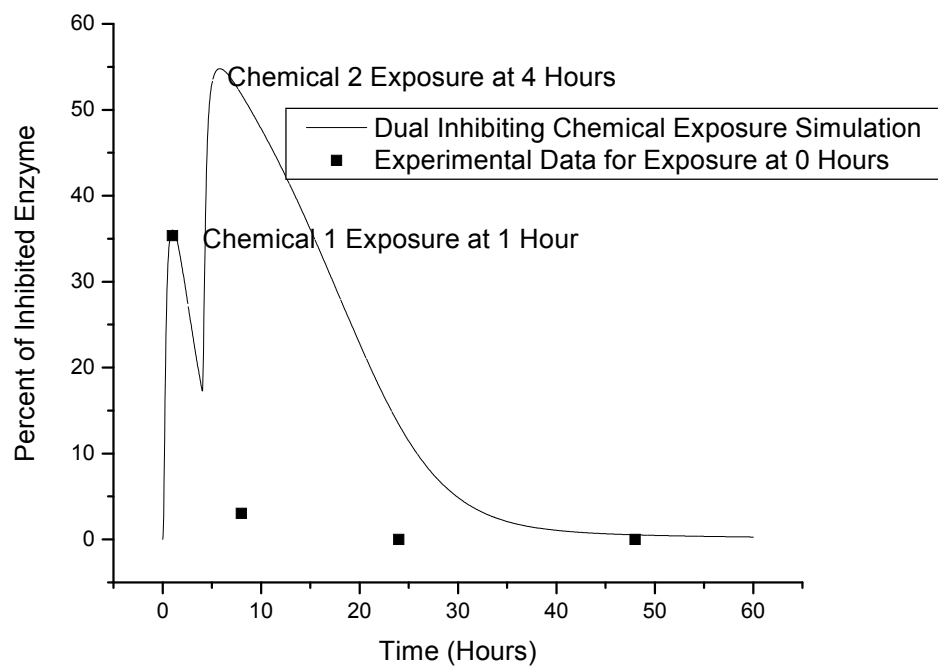
The illustrations that have been shown here demonstrate how the model can be used to estimate dose under a variety of conditions. Different exposure scenarios and different biochemical and physiologic conditions can all be tested. Conventional approaches do not afford the advantages of testing these various scenarios. Further the model can be used to test the importance of various governing factors (physiological, biochemical, thermodynamic, exposure inputs) on the ultimate relevant dose in the tissue. With the model the internal dose proximate to the impacted organ can be estimated. Models based on sound and rational science and which are well tested are very valuable and powerful tools for elucidating the impact of many factors upon the toxicologically relevant dose.

G. Experimental data needs for a PBPK/PD model for the N-methyl carbamates

Typical toxicology data collected for purposes of pesticide registration (40CFR part 158) do not include the appropriate metabolism and PK data needed to support a PBPK/PD model. Generally, metabolism studies submitted for pesticide registration include a metabolic pathway describing the parent chemical, major metabolites, and excretion products. However, these studies do not measure concentrations of parent compound and/or active metabolites in target tissue or blood but instead generally report total radioactivity in various tissues. Typical toxicology databases submitted for pesticide registration also do not include PD studies such as time to peak effect or time to recovery. With the exception of some time to recovery AChe data for a few N-methyl carbamates, there is very little appropriate metabolism or toxicology data available from pesticide registration databases for developing PBPK/PD models. EPA is actively searching the scientific literature for data and information relevant to the PBPK/PD modeling case study.

Development of PBPK/PD models is most efficient when the model developer and laboratory scientist work collaboratively and iteratively together. By first developing a preliminary model using the best available information, the modeler can communicate research needs to laboratory scientists very early in the model development process. Laboratory experiments can then be designed to directly address to the data needs. Following consultation with EPA laboratory scientists, consideration of available metabolism and mechanistic data, and consideration of the existing PBPK/PD models for several AChE-inhibiting OPs (Gearhart et al., 1990; Gearhart et al., 1994; Timchalk et al., 2002), it has been possible to develop preliminary models before conducting laboratory studies. The structures for the two models under development were provided in Figures 2 and 3. It is also notable that because of the data intensive nature of PBPK/PD models, for purposes of cumulative risk assessment, it is prudent to concentrate resources and efforts on those pesticides and exposure scenarios that are expected to contribute to the cumulative risk for a particular common mechanism group.

The types of data described below include both *in vivo* and *in vitro* experiments. For some types of information such as partition coefficients, where extrapolation from *in vitro* to the whole animal is not expected to significantly impact model uncertainty, the use of *in vitro* techniques offer an opportunity to conserve resources and to complement more resource intensive *in vivo* experiments.

The law of parsimony encourages development of the simplest model that is powerful enough to accurately predict the biological or toxicological effect of interest. Models with structures that are more complicated than necessary are difficult to interpret, tend to be over-parameterized which leads to problems with parameter identifiability, and may suggest more supporting experimental work than is really needed. The following is a discussion of what is considered to be the minimum information necessary to develop PBPK/PD models for individual N-methyl carbamates. In order to establish a linkage between PK with the AChE inhibition and subsequent recovery, it is suggested that measurements of AChE in RBC and brain are also performed with the *in vivo* PK studies described below. Additional information related to interindividual variability is also discussed below. The main focus of the following discussion, particularly the *in vivo* experiments, is on development of PBPK/PD models for rodents. The issue of scale-up and extrapolation of the rodent model to humans is considered separately (Section III.I).

1.   Types of data needed for PBPK/PD model development

'   *Chemicals of interest.* The PBPK/PD models for individual N-methyl carbamates will be developed to track parent compounds and any metabolites capable of AChE inhibition. There will be no need to explicitly track inactive species and, in the interests of model parsimony, it is preferable not to do so.

'   *In vivo AChE inhibition.* Describing the linkage between PK and AChE inhibition, is an critical aspect of the PBPK/PD modeling effort. Measurements of AChE inhibition, particularly RBC and brain, during the *in vivo* PK studies discussed below could be used to establish this linkage. Because of the rapid recovery of the N-methyl carbamates, the method used to measure AChE is an important consideration. It is suggested that *in vivo* measurements of AChE are performed using a technique, such as the radiometric method (Johnson and Russell, 1975; Hunter and Padilla, 1999; Winteringham and Fowler, 1966), that is not impacted by the rapid reversibility of the N-methyl carbamates.

'   *In vitro kinetics of AChE binding.* The reaction of an AChE-inhibiting chemical species (whether parent compound or metabolite) with AChE is described by a 2nd-order rate constant. The dissociation of the inhibitor-AChE complex is described by a 1st-order rate constant.

These parameters can be estimated using *in vitro* methods and should be obtained for blood (particularly RBC) and brain. Estimation only of the equilibrium dissociation constant, which is the ratio of the 2nd- and 1st-order rate constants, rather than the 2nd and 1st order constants themselves, would be acceptable for the purposes of developing the PBPK models. These data will be needed for all chemical species that contribute significantly to AChE inhibition.

' *Partition coefficients.* Computational algorithms are available for prediction of partition coefficients (Poulin and Thiel, 2001; Poulin and Krishnan, 1995; DeJongh et al., 1997). These algorithms provide an opportunity to develop PBPK/PD models without actually measuring partition coefficients in the laboratory. It may be prudent, however, to obtain laboratory measurements of a subset of the algorithmically-predicted values in order to determine the reliability of computationally derived partition coefficients. This determination will be particularly important if future sensitivity analyses indicate that one or more of these coefficients are important determinants in estimating exposure concentration at the site of action.

It is expected that blood:tissue partition coefficients will be required for liver, kidney, fat, slowly perfused (i.e., muscle), and brain. Blood:air partition coefficients will be needed for volatile parent compounds or metabolites. The skin:blood partition coefficient will be needed if dermal exposure is evaluated.

' *In vitro studies for metabolic clearance.* The quantitatively most important site of metabolism of N-methyl carbamates is expected to be the liver. Liver homogenates or microsomes can be used to estimate the Michaelis-Menten parameters of metabolism (Vmax: maximum rate of metabolism, Km: concentration at which the enzyme is half-saturated). Sufficient attention to the design of these experiments should enable the identification of multiple metabolic pathways characterized by different Vmax and Km values. As mentioned previously, development of PBPK/PD models is most efficient when model developers work collaboratively with laboratory scientists. If a first-generation PBPK/PD model that describes only hepatic clearance is not able to predict PK data, then screening of other tissues such as blood and kidney as potential sites of metabolic clearance may need to be considered.

The extrapolation from *in vitro* to *in vivo* can be a source of uncertainty; some limited data on compound metabolism may need to be collected *in vivo* to evaluate the suitability of the *in vitro* data.

'   *In vivo studies for metabolic parameters:*

a.   *Intravenous studies:* Analytical measurement of the time-course of the amount of parent N-methyl carbamate and AChE-active metabolites in the target tissue, site(s) of storage (if appropriate) and metabolism are suggested in addition to measurements of AChE inhibition. These datasets provide an opportunity to estimate metabolic parameters (Vmax and Km) by curve fitting without potential confounding by incorrect specification of rates of absorption from the GI tract. As noted previously, even if *in vitro* methods are used as the primary means of obtaining data on Vmax and Km, some *in vivo* data as described here should be obtained to evaluate the *in vitro* to *in vivo* extrapolation.

b.   *Oral absorption.* Absorption from the GI tract is a major route of exposure of N-methyl carbamates into the body particularly through the diet. However, estimation of oral absorption parameters is best done by fitting the model to PK data where all model parameters, except for the oral absorption parameters, have already been set.

A blood time course dataset is a good basis for estimation of oral absorption parameters. The specific oral absorption parameters to be identified will depend on the types of data available. For example, oral dosing can be administered in various ways such as corn oil gavage, water gavage, or feeding. Analytical measurement of the time course of the amount of parent N-methyl carbamate and AChE-active metabolites in blood is the minimum requirement for estimation of the oral absorption parameters. In addition, measurements in the target tissue, site(s) of storage (if appropriate), and metabolism, in addition to measurements of AChE inhibition, are suggested. (These latter measurements would be useful when considering the reliability of model predictions but they are not essential for estimation of oral absorption parameters during model development.)

'   *Interaction experiments.* When exposure occurs to two or more members of the cumulative assessment group (CAG), interactions between compounds may occur that affect PK behaviors and the associated degree of AChE inhibition. The expected sites of interaction include CYP450 and carboxyl esterases that are sites of N-methyl carbamate binding and metabolism. Development of a single PBPK/PD model for the entire CAG will require that these interactions be described explicitly in the equations of the model.

The initial approach to the development of this model will be to assume that the interactions are competitive. The mathematical description of competitive interactions is straightforward. Fortunately, the data obtained during development of the PBPK/PD models for the individual members of the CAG can also be used to parameterize competitive interaction terms for the PBPK/PD model for the entire CAG. Similarly, the individually measured binding and dissociation parameters for AChE (see *In vitro kinetics of AChE binding* above) should be sufficient to characterize the overall level of AChE inhibition for a multi-compound model. The studies conducted in support of model development for the individual compounds will thus provide much if not all of the data needed to characterize these interaction terms.

An *in vivo* multi-chemical PK and AChE inhibition study will be desirable to establish the reliability of the PBPK/PD model for at least a subset of the members of the CAG (particularly for those pesticides with the highest levels of human exposure). The preliminary PBPK/PD model for CAG as a whole can be used to design this study to ensure that dose sampling time points selection is as close to optimal as is possible. Alternatively, and in lieu of this *in vivo* study, it may be possible to design *in vitro* pharmacokinetic and AChE inhibition time course studies that can provide useful tests of the reliability of the CAG PBPK/PD model.

' *Other additional information.* As noted above, development of a PBPK/PD model is a process that iterates between model development at the computer terminal and targeted laboratory experiments. The data collection needs identified above should provide robust PBPK/PD models sufficient to the task of predicting AChE inhibition for various exposure scenarios. Some kinds of additional data, however, may serve to increase confidence in the model to an even greater degree and to provide additional capabilities relevant to risk assessment.

Data on compound elimination in urine, feces, and exhaled breath would support the model development, and test the accuracy of the assumptions concerning mass balance. Demonstration that the PBPK model equations accurately simulates the results of a mass balance study increases the level of confidence in the model structure, and in the use of its predictive capability for risk assessment.

Data on interindividual variability in PBPK/PD model parameters, in PK behavior and in AChE inhibition would support a "Monte Carlo" analysis. In a Monte Carlo analysis, PBPK models parameter values are described by statistical distributions rather than by single,

fixed data points. At each run of the model, random samples are taken from the distributions to create a new set of parameter values. Each run of the model can thus be thought of as representing a different individual in a population (USEPA 1996 and 1997). Thus, a Monte Carlo analysis supports characterization of variability and uncertainty in the estimates of AChE inhibition based upon the uncertainty and variability in the parameters. A Monte Carlo analysis would first be performed on the PBPK model for rodents. Reparameterization of the PBPK model to humans would require consideration of the issues addressed in the discussion on interspecies extrapolation (see Section III.I) with additional concern for how parameter variability scales between species. The Monte Carlo model would provide a capability for prediction of population variability in AChE inhibition This capability would help address the uncertainty associated with interindividual variability.

PBPK/PD models are capable of incorporating specific information relating to sex, age, and/or other factors that impact the manner in which individuals are affected by chemical exposures. The datasets used to support development of a PBPK/PD model are typically collected in a sex-specific manner. Some sex-specific differences in parameter values are well known, for example the differences in hepatic CYP450 activities, the model has an associated "sex" which is defined by the supporting datasets. Sex-specific differences in key parameter values, such as rates of bioactivation or detoxification, could be associated with measurable differences in degrees of AChE inhibition for the same exposure. Once the initial PBPK/PD model is developed for males or females, a sensitivity analysis could be conducted to determine the degree to which model-predicted AChE inhibition is sensitive to sex-related changes in key parameter values. This sensitivity analysis would provide a good indication of whether or not collection of additional data in the other sex is necessary.

PBPK/PD models are usually developed using datasets from adult animals. Information related to identification and description of sensitive life stage(s), such as maturation profiles for critical metabolic pathways, would also be helpful in developing the PBPK/PD models. It is possible to incorporate into these models descriptions of how parameter values change with age. This information allows the model to describe how PK behaviors change with age, as with the transition from childhood to adulthood. These capabilities depend on the availability of adequate supporting datasets, such as growth curves for the major tissue groups in the body, and age-dependence in the activities of enzymes that activate or clear the chemicals of interest. In the absence of such information, the PBPK/PD model is typically developed for the adult

and health-protective, default approaches are invoked to account for potential age-dependent pharmacokinetic behaviors contribute to the age-dependence of risk.

2. Uncertainty associated with availability of appropriate data

The Agency has not yet determined which method or methods that will be used to develop the cumulative risk assessment for the N-methyl carbamates. However, the availability of appropriate data is expected to impact the level of refinements and type of method(s) used. At this time, it is unknown whether or not sufficient data will be available to the Agency for model development or for evaluating the reliability of the PBPK/PD models for the N-methyl carbamate pesticides in a timely manner for the expected release of the preliminary cumulative risk assessment in 2005. However, one of the goals of the current research effort and case study is to consider the degree to which completeness and availability of appropriate PK data impacts model uncertainty when developing PBPK/PD models for use in regulatory settings. As part of the case study, in the future, the Agency will also carefully evaluate the application of uncertainty, extrapolation, and safety factors, particularly the FQPA 10x factor for infants and children, when using PBPK/PD models to estimate cumulative risk. The Agency anticipates further discussion and consideration of overall uncertainty related to data availability in the future as work on the case study continues.

H. Model Evaluation and Quality Control.

An essential part of the modeling process is model evaluation: the process of determining the degree to which a model satisfies the needs that led to the model's creation. Model development is an iterative process in which model creation (i.e., when important aspects of biology are captured in mathematical and computer models) alternates with model evaluation (i.e., when the model is tested and challenged with data and analysis). Any failures in the evaluation phase are used to identify inadequate approximations and faulty biological assumptions, which can then be corrected in a new model creation phase. When it comes time to use the model, the process must end with an evaluation step. The following process is a sequence of steps whose application is intended to increase confidence that the PBPK/PD models are reliable tools for assessing risk. In many ways, the sequence parallels the process of model creation. This approach has been treated more generally by Clark, et al. (2003, *in prep*).

1. Model Purpose

The specific use for the model must be explicitly defined *before* an evaluation of model suitability can be begun. The purpose of the model constrains its structure and determines the details of the rest of its evaluation. Some examples of questions that must be answered here are:

Does the model need to predict AChE inhibition or parent and metabolite concentrations? In which tissues must the model predict these values? What metrics, such as area under the curve, peak value, or area under the curve above a threshold value, must be computed? What sorts of exposure inputs are required? How precisely must the model predict the different outputs?

2.   Biological Characterization and Model Structure

Although the quantitative machinery gets much of the attention, in fact a PBPK/PD model is initially a narrative statement of biological descriptions and hypotheses. This narrative must include the general biological features that affect concentrations of parent chemicals and the relevant metabolites, and also sources and the nature of intra- and inter-individual variability. PBPK/PD models include a number of approximations and assumptions that should be made explicit. Some aspects of model structure, such as the treatment of tissues as well-mixed compartments, concentrating all or most of metabolism in one or two tissues, and the lumping of compartments with similar characteristics, are common to most PBPK/PD models. Other aspects of model structure are specific to the chemicals being considered, such as simplifications of metabolic pathways, and the nature of protein binding. Still others are specific for particular endpoints, like the description of AChE synthesis, degradation, and inhibition.

This largely qualitative description of the biological and toxicological profile(s) for the chemical(s) of interest should be reviewed in the context of the relevant scientific literature. In cases where the literature is unclear about the details needed for modeling, and/or for which the literature supports more than one descriptions of a particular feature, it should be possible, through modeling those alternatives, to quantify the degree to which the ambiguity matters for the particular endpoints of concern.

3.   Mathematical Descriptions

It is convenient when assessing a PBPK/PD model to abstract its formal mathematical description from its implementation in a particular programming language. Much of that description is well-established (Ramsey and Andersen, 1984): the mathematical forms for perfusion-limited and diffusion-limited compartments are well-known, for instance. Other aspects are not so well-established, such as protein binding, absorption from the GI tract, dermal absorption, and submodels for AChE inhibition and regeneration. Some mathematical descriptions of biological features, such as receptor binding or protein binding more generally, may carry with them assumptions about how rapidly concentrations of ligand, receptor, and the ligand-receptor complex come to steady state relative to the other temporal changes in the system. For completeness, it is

desirable that the mathematical description includes the nature of inter- and intra-individual variability, including measurement error. Details of the probability model evaluated here, such as particular probability distributions used will often depend upon the context within which the model is used, such as experimental design or the risk assessment scenario, but this part of the model description is essential for proper statistical treatment of parameter estimation.

## 4.	Computer Implementation

PBPK/PD models are typically written in a specialized high-level language, which is itself a complex computer program, very often a proprietary language whose source code is usually hidden from the ordinary user. Thus, the reliability of the software in which the model is written although an important concern, is one that will have been addressed in a larger context. Thus, the primary concern in this phase of model evaluation is that of the code for the model itself.

An advantage of analyzing PBPK/PD models first into a mathematical description prior to a computer implementation of that description is the opportunity to evaluate the mathematical description and computer implementation separately.   Evaluation at this step involves checking of computer code against mathematical description and also checking that features of the language, such as integrator options, are used correctly (*e.g.*, stiff methods used for systems that are likely to be stiff, *etc*.)

Details of the evaluation depends upon language. For languages such as MCSim and ACSL, which are essentially algebraic languages, the form of the model description follows closely that of the mathematical description. For many PBPK/PD models, like that shown in Figure 3, details of statement syntax and the use of the proper variables in a model that may have hundreds of similar-looking variable names becomes a critical and difficult part of the evaluation. ERDEM, on the other hand, is essentially a pre-coded PBPK model for a large number of potential compartments and chemicals. The specific implementation for a particular set of chemicals is based on limiting that general model by the use of switches and implementation-specific parameter values. For this sort of model, review consists of making sure the switch settings and parameter values correspond to the intended model. Again, the large number of parameters necessitated by a model for multiple exposures makes this a tedious part of the model evaluation; though a good user interface can facilitate the review significantly by making it easier to group related variables together.

A valuable approach for complex models is separate, independent implementation of the same mathematical description in separate languages. Greater confidence in the software implementation is achieved when two independent implementations of a model give numerically similar results to the same inputs. Two models for the N-methyl carbamates are being developed, in ERDEM and in MCSim. This allows the software implementations to be directly tested through comparisons of their respective outputs given the same input. In addition, dual model implementation provides the opportunity to take advantage of the unique features of each language, such as the advanced statistical features of MCSim and the ability for rapid prototyping of alternative models in ERDEM.

5.   Parameter Analysis and Quality of Model Fit

Model parameters can be grouped into several categories: physiological parameters such as tissue volumes, blood flows, AChE synthesis and degradation rates; chemical-specific parameters such as partition coefficients, metabolic rate constants, coefficients for protein binding, coefficients for AChE inhibition; and parameters for determining the stochastic behavior of model, such as inter-individual variances of the true parameter values. Their values are determined in different ways. Physiological parameters are usually determined by combining body weight with tabulated relationships between body weight and the other physiological parameters (e.g., Brown, et al., 1997). Chemical-specific parameters may be estimated by fitting the model to data, as is often the case with metabolic parameters. In some cases, algorithms exist for estimating them from other chemical-specific characteristics, such as approaches for estimating partition coefficients from octanol:water partition coefficients (e.g., Poulin and Krishnan, 1995; Poulin and Thiel, 2001). Parameters for the stochastic characteristics of the model may be estimated from experimental data by fitting the model directly. However, especially for human stochastic parameters used for predicting human variability, these parameters may be inferred from studies of the degree to which people vary in, for example, relevant biochemical parameters such as enzyme activities.

The first step in evaluating the quality of the parameters used in the model is to affirm the correctness and relevance of particular values by reference to literature values. Once this is completed, it is important to determine the critical parameters important for model outputs relevant to the risk assessment as well as for data sets used to evaluate model reliability. This determination helps identify parameters whose values are unreliable, because they have not been "tested" in the comparisons between model predictions and new datasets, and identifies parameters whose values are particularly important, and thus warrant closer examination. Engineering tools, such as sensitivity analysis, and tools from

statistical experimental design for non-linear model parameter estimation, are useful for identifying the parameters that are most critical for determining the values of model outputs.

Also important is the ability of the model to predict results in data sets that have not been used to estimate parameters. The closer the experimental designs of such datasets are to the regimen in which the model will actually be used, the more reliable the results of such evaluation will be for predicting the utility of the model. It is important to note that strict goodness-of-fit testing is not necessarily the right approach, here. The null hypothesis on which goodness-of-fit testing is based, that the model under consideration is the true model that generated the particular experimental data set, is clearly false *a priori*. Failure to reject a goodness-of-fit test means no more than that the experimental design was inadequate to detect the deviation of the PBPK/PD model from the truth, but does not quantify how close the model is to the true model. Instead, the degree to which the model fails to predict experimental results, in terms of readily interpretable metrics such as percent error of prediction, with a measure of its uncertainty, is a more useful measure. It is preferred to consider the appropriate the level of precision at the beginning of the modeling effort, perhaps during Stage 1 (definition of model purpose).

I.   Model scale-up and extrapolation from rodents to humans.

Use of laboratory animals to study N-methyl carbamate toxicology is justified by the assumption that the data so obtained are relevant to humans. With respect to PBPK/PD modeling, this means that we assume that the model structure that describes N-methyl carbamate PK and AChE inhibition in rats is also appropriate for humans. This assumption is consistent with data on the common role of AChE in the rodent and human nervous systems as well as with other data on N-methyl carbamates. The problem of scale-up of the model from rats to humans thus becomes one of identifying appropriate human values for the model parameters. The scaling behaviors of all of the parameters of the rodent model should be considered in this process. Identification of a full set of human parameter values allows the model to be used for prediction of AChE inhibition in people. In practice, most if not all of the data available to support model development will be obtained from rodents and rodent tissues. It is a certainty that the database of human information for PBPK/PD model development will be minimal compared to that available for rats. Scaling and extrapolating the PBPK/PD model from rats to people will thus involve a number of steps:

1.   Use of human data when available and if appropriate. For example, data on human physiological values such as tissue volumes and blood flows can be used directly in the model. Chemical-specific data that can be obtained from *in vitro* studies from human tissues, such as partition coefficients, rates of metabolism, and interactions with AChE, would be valuable. The

ability to obtain such *in vitro* data depends on the availability of appropriate human tissue samples, such as hepatic microsomes and blood.

2.   Identification of any relevant and appropriate non-human primate data and evaluation of its possible use as surrogate for human data.

3.   In the absence of relevant human information, allometric scaling of the rodent parameter are values. This approach defines the parameter values as functions of body weight. For example, the breathing rate is scaled from rodents to humans as a fractional power (usually 0.75) of body weight (BW). Tissue volumes, on the other hand, are usually scaled as $BW^{1.0}$.

4.   Use of rodent parameter values directly in the human model. In the absence of relevant human data and a lack of information on how to allometrically scale a parameter value, the most judicious approach may be to use the rodent value directly, i.e., without change, in the human model. Oral uptake absorption rate constants, for example, may be treated in this manner. As with all approaches to scale-up, the contribution to overall model uncertainty of this approach should be considered.

At present time, the PBPK/PD models under development for the N-methyl carbamate pesticides have not been scaled from rodents to humans. However, it is expected that when faced with uncertainty about the appropriate scale-up for specific parameters, choices can often be identified that are health protective. For example, if the appropriate scale-up of oral absorption rate constants is not clear, then the option that maximizes the rate of absorption in the human model could be used, since this approach is expected to maximize the inhibition of AChE predicted by the human model. This approach will cause the human model to over predict human health risk in the face of uncertainty about parameter scale-up. As the case study develops further, the Agency will provide the sources of data and relevant scaling of all parameters so as to make the PBPK/PD modeling effort transparent and open for scientific evaluation.

The approach to scale-up described here will produce a human version of the PBPK/PD model. Expert judgment will be required to evaluate the degree of uncertainty associated with the scale-up with specific attention paid to the question of how the uncertainties associated with the human PBPK/PD model compare to the uncertainties of an empirical modeling approach.

IV.   SUMMARY

The current document outlines the on-going work by EPA to develop a strategy for performing cumulative risk using PBPK/PD modeling. A case study with AChE-inhibiting N-methyl carbamates pesticides is under development. EPA is proposing to perform a dual modeling effort in this case study. By developing models in two different programming languages the advantages of each platform can be put to use. The generation of two models is also expected to provide an additional level of quality control

for error detection and correction. Incorporating PK and mechanistic data into risk assessments is expected to improve the biological and scientific basis for the assessments and is thus expected to improve regulatory decisions. Although using PBPK/PD models is likely to reduce the overall uncertainty for a particular risk assessment, it is not intended to remove all uncertainty and may actually highlight areas of uncertainty that were not previously considered or evaluated. The key consideration in evaluating the utility of PBPK/PD models is not that the model be correct in any absolute sense but rather that it be arguably better, i.e., less uncertain, than an alternative empirical approach or use of default assumptions. In the future, EPA will need to critically evaluate the balance between model uncertainty particularly when associated with incomplete PK and/or mechanistic data sets with the reduction in overall risk assessment uncertainty associated with utilizing these types of models and information. As appropriate PK data become available, the Agency will also address some technical issues in the case study such as parameter estimation and sensitivity analysis not considered in depth in the current document. Since the passage of FQPA (1996), the Agency has taken a step-wise approach to developing its cumulative risk assessment methodologies and risk assessments. The current document is a continuation of this step-wise approach. Further scientific review is anticipated in the future as the case study and PBPK/PD strategy are developed.

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